

subtilis (ATCC 6633),¹ test organism H, prepared as described in § 436.103 of this chapter, using the method described in paragraph (b)(2) of that section.

(b) *Preparation of plates*—(1) *Baselayer*. Add 42 milliliters of medium 2 described in § 436.102(b)(2) of this chapter to each Petri dish (25 millimeters x 150 millimeters) and allow to harden on a flat, level surface. To prevent condensation of excess moisture, raise the tops slightly while the agar hardens.

(2) *Seed layer*. Melt nutrient agar medium 2 described in § 436.102(b)(2) of this chapter. Accurately measure a sufficient quantity of the melted agar, cool to 48° C., and add the appropriate quantity of the spore suspension prepared as described in paragraph (b)(7)(ii)(a) of this section. Swirl the flask of inoculated agar to obtain a homogeneous suspension. Add 8 milliliters of this inoculated agar to each plate, spread evenly, and allow to harden on a flat, level surface. For accurate results, it is necessary to obtain uniform distribution of the agar over the entire surface of the plates.

(c) *Assay*. For each spot on the paper described in paragraph (b)(7)(i)(c) of this section, cut a strip 1.5 centimeters by approximately 14 centimeters with the center of each strip centered about the line of descent of the spot. Place all strips on plates with the aid of forceps within as short a period of time as possible. Use maximum spacing between strips. Insure complete contact so that the entire strip becomes uniformly moistened. Allow to stand for 30 minutes. Remove the strips and identify each strip location on the Petri dish. Incubate the plates for 16–18 hours at 37° C. Any zone of inhibition corresponding to factor A in the sample must not be greater than that of the 0.2 milligram-per-milliliter factor A standard. Also, the two areas of inhibition for the sample due to the presence of factor A and vancomycin must compare to the corresponding two areas of inhibition of the known mixture in their respective distances from their origins.

(8) *Heavy metals*. Proceed as directed in § 436.208 of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.86 Vancomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Vancomycin is a tricyclic glycopeptide. It is a free flowing white to off-white colored powder. It is so purified and dried that:

(i) It contains not less than 925 micrograms of vancomycin per milligram, calculated on the anhydrous basis.

(ii) It contains not less than 92 percent vancomycin factor B and not more than 3 percent of any individual vancomycin related factor.

(iii) Its moisture content is not more than 20 percent.

(iv) Its heavy metals content is not more than 30 parts per million.

(v) It gives a positive identity test for vancomycin.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, chromatographic purity, moisture, heavy metals, and identity.

(ii) Samples required: 12 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed sample of approximately 100 milligrams in a 100-milliliter volumetric flask and dissolve in approximately 50 milliliters of distilled water and 1.0 milliliter of 0.1N hydrochloric acid. Swirl or sonicate to dissolve the sample and bring to volume with distilled water. Further dilute an aliquot of this solution with 0.1M potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) *Chromatographic purity*. Proceed as directed in § 436.366 of this chapter. The

¹Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

relative amount of vancomycin B is not less than 92 percent, and the relative amount of any related substance is not more than 3 percent.

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *Heavy metals*. Proceed as directed in § 436.208 of this chapter.

(5) *Identity*. Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc preparation as described in § 436.211(b)(1).

[59 FR 8400, Feb. 22, 1994]

§ 455.88 Rifabutin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Rifabutin is an amorphous red-violet powder. It is (9S,12E,14S,15R,16S,17R,18R,19R,20S,21S,22E,24Z)-

6,16,18,20-tetrahydroxy-1'-isobutyl-14-methoxy-

7,9,15,17,19,21,25-heptamethylspiro[9,4-(epoxypentadeca

[1,11,13]trienimino)-2H-furo[2',3':7,8]naphth[1,2-d]imidazole-2,4'-piperidine]-5,10,26-(3H,9H)-trione-16-acetate. It is very slightly soluble in water, sparingly soluble in ethanol, and soluble in chloroform and methanol. It is so purified and dried that:

(i) Its potency is not less than 950 micrograms and not more than 1,020 micrograms of rifabutin activity per milligram on an anhydrous basis.

(ii) Its content for the four major related substances detected by high-performance liquid chromatography (HPLC) is not more than 1.0 percent each. All other unknown related substances are not more than 0.5 percent. The total of all related substances is not more than 3.0 percent.

(iii) Its moisture content is not more than 2.5 percent.

(iv) Its *N*-isobutylpiperidone content is not more than 0.5 percent.

(v) It gives a positive identity test.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for rifabutin potency, related substances, moisture, *N*-isobutylpiperidone, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 ± 1 nanometers, an 11 centimeters X 4.7 millimeters (i.d.) column packed with microparticulate (5 to 7 micrometers in diameter) packing material such as octylsilane chemically bonded to porous silica (U.S. Pharmacopeia designation L7), a flow rate of about 1.0 milliliter per minute, and a manual or automatic injector capable of injecting 10 microliters. The retention time for rifabutin is between 9 and 11 minutes. Reagents; working standard, sample, and resolution solutions; system suitability requirements; and calculations are as follows:

(i) *Reagents*—(A) *Hydrochloric acid*, 2*N*. Dilute 85 milliliters of hydrochloric acid (37 percent) with distilled water to 500 milliliters.

(B) *Potassium dihydrogen phosphate*, 0.1*M*. Prepare a solution containing 15.4 grams of potassium dihydrogen phosphate monohydrate (potassium phosphate monobasic) per liter of distilled water.

(C) *Sodium hydroxide*, 2*N*. Dissolve 8 grams of sodium hydroxide pellets in 100 milliliters of distilled water.

(D) *Mobile phase*. Acetonitrile:phosphate buffer, pH 6.5, 50:50. Mix equal quantities of acetonitrile and 0.1*M* potassium dihydrogen phosphate and adjust to an apparent pH of 6.5 ± 0.1 by dropwise addition of 2*N* sodium hydroxide. Filter through a suitable filter capable of removing particulate matter 0.5 micron in diameter and degas it just prior to its introduction into the chromatograph. Slight adjustments of the mobile phase components ratio may be made in order to meet the system suitability requirements described in the system suitability tests in paragraph (b)(1)(iii) of this section.

(ii) *Preparation of working standard, sample, and resolution test solution*—(A)